AMERICAN ACADEMIC PUBLISHER

INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

MOLECULAR DOCKING ANALYSIS TO EVALUATE THE WOUND HEALING POTENTIAL OF CALANHOE DAIGREMONTIANA EXTRACT

Mirxalikova M.B[1], Mamadrahimov A.A[1], Jalilov M.K[2], Abduxoliqov F[2], Kuchkarov N.Y[3]

[1]PhD Student, National University of Uzbekistan, Tashkent, Uzbekistan
[1]Institute of Bioorganic Chemistry, Tashkent, Uzbekistan
mirxalikovamaftuna6502@gmail.com

Abstract

The purpose of this work is to study the extracted formation of Calanhoe Daigremontiana, to establish their flavonoid formation, to determine the physicochemical characteristics and wound-healing activity. The experimental object of the study: in order to study pharmacological activity, Calanhoe Degremontiana plant was used, collected during the winter period in January 2026 on the territory of the Republic of Uzbekistan (Tashkent). Flavonoid composition was determined by using High Performance Liquid Chromatography – Diode Array Detector (HPLC-DAD). The molecular docking method was used to examine the active components based on their ability to interface with the target protein.

Keywords

Calanhoe Daigremontiana, Wound Healing activity, HPLC-DAD, Molecular Docking,

Introduction. Kalanchoe daigremontiana, commonly known as Mother of Thousands, is a unique and intriguing succulent that has captured the hearts of plant enthusiasts worldwide. This remarkable plant, native to Madagascar, is famous for its ability to produce hundreds of tiny plantlets along the edges of its leaves, earning it its evocative common name. It belongs to the Crassulaceae family of stonecrops. The French Botanical Society Members Madame and Monsieur Daigremont are honored with the specific epithet, daigremontiana. Well-known medicinal herbs called kalanchoe species (with a rich chemical composition that includes bufadienolides and flavonoids) are utilized as analgesics and anti-inflammatory treatments in traditional medicine [1][2][3].

Flavonoids are a family of naturally occurring phenolic chemicals that are produced in plants as bioactive secondary metabolites. These molecules are what give plants their taste, color, and pharmacological properties [4][5]. They are strong antioxidants that shield plants from adverse environmental circumstances. Studies has shown the immunomodulatory, anti-inflammatory, anticancer properties and also, different biological activities of flavonoids [6]. Numerous biological functions of flavonoids derived from natural sources have been investigated so far, including cytotoxic, cytoprotective, ATPase, anticancer, anti-inflammatory, antioxidant, and analgesic properties but wound healing activity has not been fully studied.

Materials and Methods.

Plant Material: The whole plant of Calanhoe Daigremontiana was collected from Biology faculty at National University of Uzbekistan in Tashkent.

Chemicals requirement: The chemicals and reagents used in the project work which are taken from Institute of Bioorganic Chemistry.

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

Preparation of Extract: The well condition of Calanhoe Daigremontiana was collected by using knife and hand-picking method. The plant should then be cleaned with fresh water to get rid of any foreign materials. The whole plant body is crushed in a mortar and pestle and taken 1 gr of crushed plant extraction with Ethanol solvents using Ultrasonic (Bio base) at 25 ^{0}C for 15 minutes. The process is executed 2 times. The obtained extract was filtered for examination in High Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD).

The active compounds present in the ethanolic extract of Calanhoe Daigremontiana were identified and confirmed by various qualitative, phytochemical investigations such as flavonoids and corresponding biological activities of those compounds have supported in the process of wound heling. The fresh extract was subjected to HPLC-DAD studies.

HPLC-DAD analysis:

According to the data presented in the literature, in the processes of determination of compounds belonging to phenolic compounds by the HPLC method, buffer systems prepared from phosphoric, acetate, and trifluoroacetic acid and acetonitrile were used as eluents. However, we used a trifluoroacetic acid buffer system and acetonitrile to test this sample [6].

In order to study the flavonoid composition of the extracted Calanhoe Daigremontiana, High-Performance Liquid Chromatography with Diode Array Detector was used under the following conditions:

-Agilent-1260 chromatograph (equipped with an autosampler),

-Poroshell 120 EC-C 18, 4 μm, 4.6x150 mm column,

-Absorption (wavelength) –254, 269 nm,

-Thermostat (column) temperature 30 ^{0}C,

-Flow rate – 0.8 ml/min.

C-acetonitrile, D-0.1% trifluoroacetic acid buffer (pH=3). The buffer concentration gradient with acetonitrile was checked in the solvent system with conditions: 0–2 min – acetonitrile 2% (v/v), 2–42 min – acetonitrile 42% (v/v), 42–45 min – acetonitrile 2% (v/v).

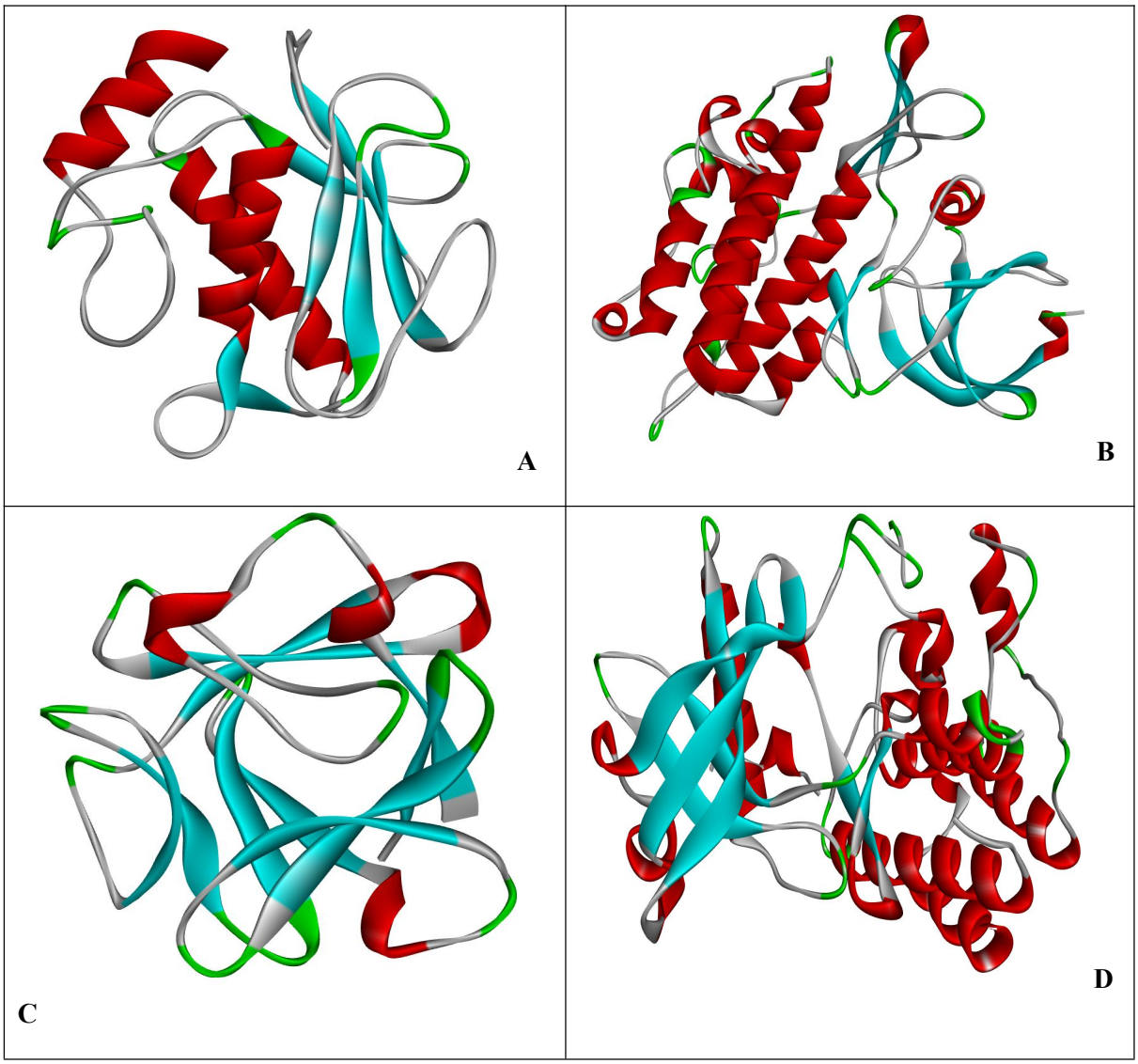
MOLECULAR DOCKING ANALYSIS

Ligands: Ligands were carefully selected based on their reported biological relevance and potential activity against the target proteins. The three-dimensional (3D) structures of the selected ligands were retrieved from the PubChem database in Structure Data File (SDF) format. Prior to docking, the ligands were prepared by performing geometry optimization, addition of hydrogen atoms, and assignment of appropriate partial charges to ensure accurate molecular representation and reliable docking results.

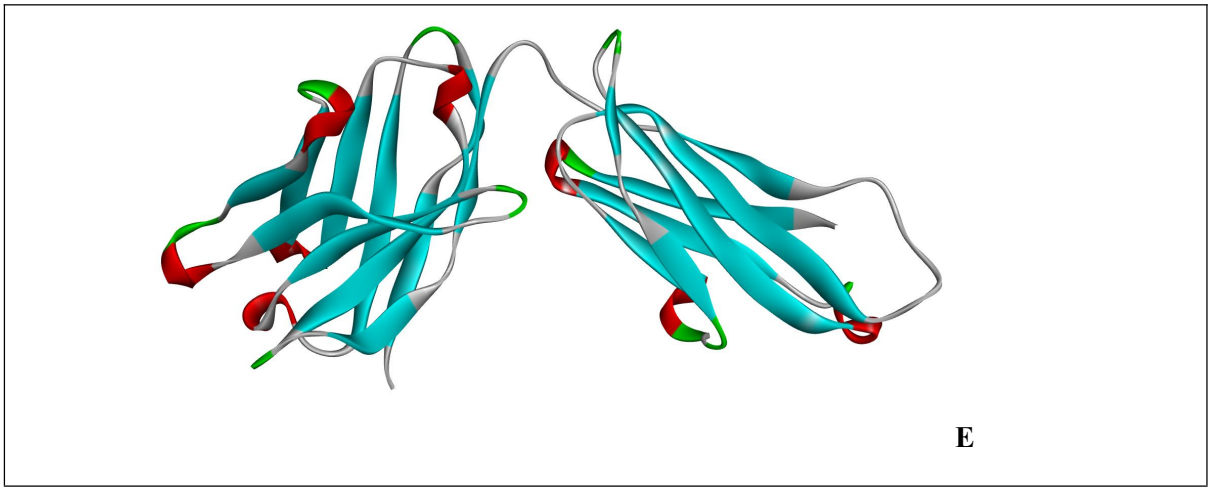
Protein Target: The three-dimensional (3D) crystal structures of the target proteins were retrieved from the Protein Data Bank (PDB). Prior to molecular docking, all protein structures underwent a rigorous preprocessing protocol to ensure structural integrity and reproducibility. This included the

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

removal of crystallographic water molecules and non-essential heteroatoms, followed by the addition of missing hydrogen atoms and correction of bond orders where necessary. Subsequently, the proteins were subjected to energy minimization to relieve steric clashes and optimize geometric conformations. The study focused on five biologically relevant proteins: matrix metalloproteinase-9 (MMP-9; PDB ID: 1GKC), transforming growth factor-beta (TGF-β; PDB ID: 1PY5), fibroblast growth factor-2 (FGF-2; PDB ID: 1BFB), vascular endothelial growth factor (VEGF; PDB ID: 3HNG), and interleukin-6 (IL-6; PDB ID: 4CNI).



AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026



(A) Docking with MMP9 (PDB ID: 1GKC), (B) Docking with TGF-beta receptor I kinase (PDB ID: 1PY5), (C) Docking with Fibroblast Growth Factor (PDB ID: 1BFB, (D) Docking with VEGFR1 (PDB ID: 3HNG), (E) Docking with IL-6 (PDB ID: 4CNI).

LIPINSKI'S RULE: 48,49

The drug-likeness and oral bioavailability potential of the selected ligands were evaluated according to Lipinski's Rule of Five (Ro5). This rule identifies the physicochemical boundaries—Molecular Weight (MW < 500 Da), Hydrogen Bond Donors (HBD < 5), Hydrogen Bond Acceptors (HBA < 10), and Lipophilicity (LogP < 5)—that often correlate with good oral absorption.

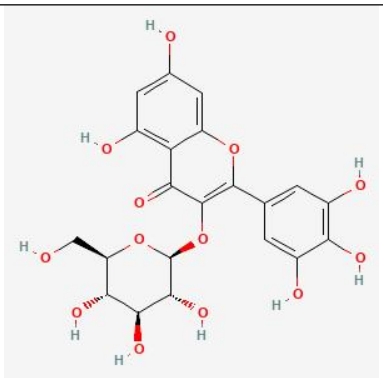
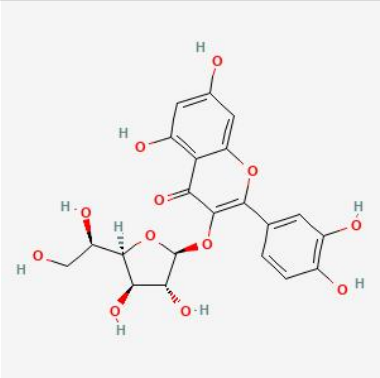
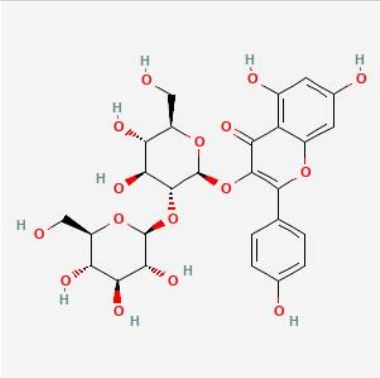
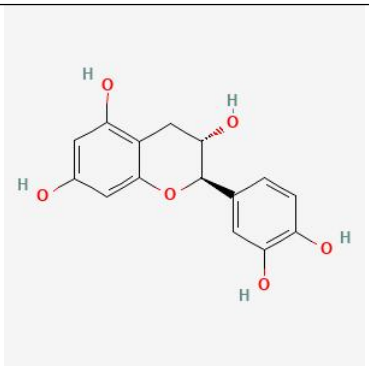
Table 3: Structure of ligands in ethanolic extract of Calanhoe Degremontiana

S.No	Name of Ligands	Structure
1	Rutin	

AMERICAN ACADEMIC PUBLISHER

INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

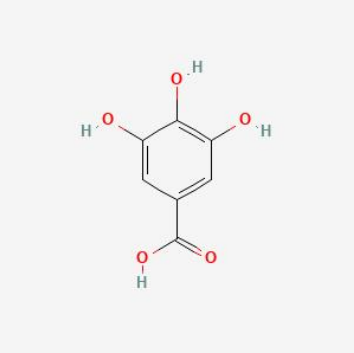
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

2	Myricetin-3-O-glucoside	
3	Isoquercitrin	
4	Kaempferol sophoroside	
5	(+)-Catechin	

AMERICAN ACADEMIC PUBLISHER

INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

6	Gallic acid	

Results and Discussion:

We have sequentially carried out the consequence of Calanhoe Daigremontiana plant extract of HPLC-DAD analysis, and molecular docking assement for wound healing effect.

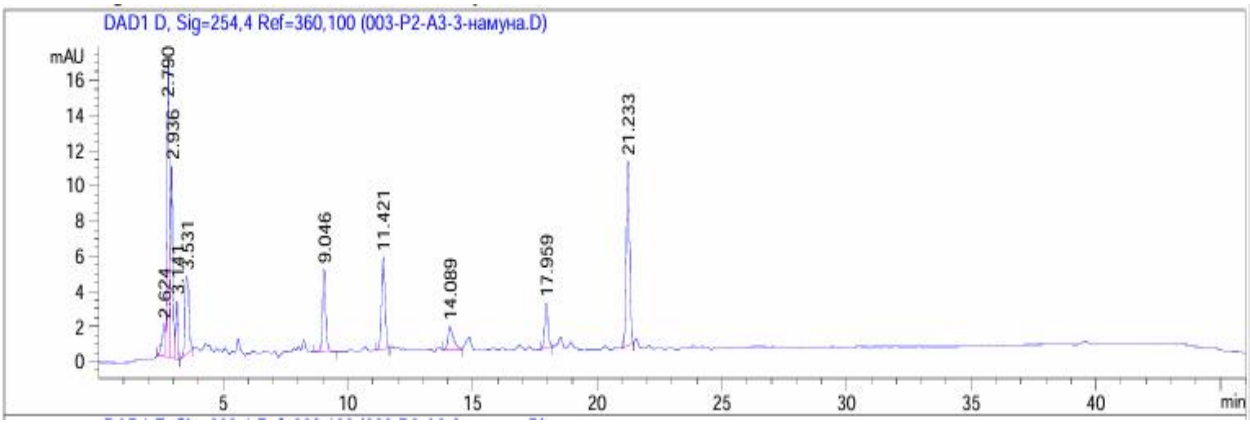


Figure 1: HPLC-DAD analysis for ethanolic extract of Calanhoe Daigremontiana extract

Table 1: Ligands of ethanolic extract of Calanhoe Daigremontiana

No	Compound name	% of Peak area	Retention time	Molecular formula
1	Rutin	20.5287	21.233	$C_{27}H_{30}O_{16}$
2	Myristicin-3-O-glycoside	5.0356	17.959	$C_{11}H_{12}O_3$
3	Isoquercitrin	3.9438	14.089	$C_{21}H_{20}O_{12}$
4	Kaempferol	10.6047	11.421	$C_{15}H_{10}O_6$
5	Catechin	8.7981	9.046	$C_{15}H_{14}O_6$
6	Gallic acid	10.2321	3.531	$C_7H_6O_5$

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

Small Molecules: Among the tested compounds, (+)-Catechin and Gallic acid demonstrated the highest adherence to Lipinski's criteria. (+)-Catechin, with a molecular mass of 290.27 Da and optimized H-bonding capacity, fully satisfies the drug-likeness parameters. Gallic acid also fits well within the oral bioavailability space, although its molar refractivity (39.47) is slightly below the standard range.

Table 2. Evaluation of Physicochemical Properties and Lipinski's Rule of Five

LIGANDS	Lipinskis rule				
	Molecular mass (<500)	Hydrogen bond donor(<5)	Hydrogen bond acceptor(<10)	High lipophilicity (<5)	Molar refractivity (40-130)
Rutin	610.52	10	16	1.58	141.38
Myricetin-3-O-glucoside	482.39	9	13	1.24	109.16
Isoquercitrin	464.38	8	12	1.55	110.16
Kaempferol sophoroside	610.52	10	16	1.81	140.52
(+)-Catechin	290.27	5	6	1.33	74.33
Gallic acid	170.12	4	5	0.21	39.47

Glycosylated Flavonoids: Compounds such as Rutin, Myricetin-3-O-glucoside, Isoquercitrin, and Kaempferol sophoroside showed several violations of the Ro5. Specifically, Rutin and Kaempferol sophoroside exceeded the thresholds for molecular mass (> 600 Da), H-bond donors (10), and H-bond acceptors (16).

Interpretation of Violations: The multiple violations observed in these larger glycosides (Rutin and Kaempferol derivatives) are primarily due to their complex sugar moieties, which increase hydrophilicity and molecular size. While these violations suggest potential challenges for passive membrane permeability, it is important to note that many natural polyphenols remain bioactive through active transport mechanisms or metabolic breakdown into smaller aglycones in the gastrointestinal tract.

In summary, while the smaller phenolic compounds show excellent drug-likeness profiles, the larger glycosides may require specific delivery systems or further investigation into their metabolic pathways to ensure effective systemic exposure.

Table 5: Dock Binding energies of the ligands interact with proteins

		FGF-2	TGF-	MMP-9	VGEF	IL-6

AMERICAN ACADEMIC PUBLISHER

INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

	LIGANDS	(1BFB)	β(1PY5)	(1GKC)	(3HNG)	(4CNI)
		Kcl/mol	Kcl/mol	Kcl/mol	Kcl/mol	Kcl/mol
A	Rutin	-7.3	-9.6	-8.8	-8.4	-8.3
B	Myricetin-3-O-glucoside	-6.4	-10.1	-7.4	-7.7	-7.9
C	Isoquercitrin	-5.6	-9.5	-8.8	-8.4	-7.3
D	Kaempferol sophoroside	-6.2	-9.5	-7.3	-8.2	-7.2
E	(+)-Catechin	-5.8	-9.5	-9.7	-8.3	-7.6
G	Gallic acid	-4.7	-6.5	-6.4	-6.1	-6.6

1. Interaction between ligands with MMP-9 (1GKC). The molecular docking analysis revealed that (+)-catechin exhibited a strong binding affinity toward matrix metalloproteinase-9 (MMP-9) with a binding energy of −9.7 kcal/mol. The interaction profile indicates that the ligand is well accommodated within the active site of the protein through multiple stabilizing interactions.

Specifically, conventional hydrogen bonds were observed with key amino acid residues such as Tyr420 and Ala191, which contribute significantly to the stability of the ligand–protein complex. Additionally, π–cation and π–anion interactions involving residues such as His411 and Glu402 further enhance binding affinity. Hydrophobic interactions, including π–alkyl and π–π stacking, were identified with residues such as His401, Leu188, and Val398, reinforcing ligand stabilization within the binding pocket.

Furthermore, several residues, including Pro421, Met422, Ala189, and His405, were involved in van der Waals interactions, contributing to the overall binding conformation and complementarity between the ligand and the receptor.

Overall, the combination of hydrogen bonding, electrostatic, and hydrophobic interactions suggests that (+)-catechin forms a stable and energetically favorable complex with MMP-9, highlighting its potential as an effective inhibitor.

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

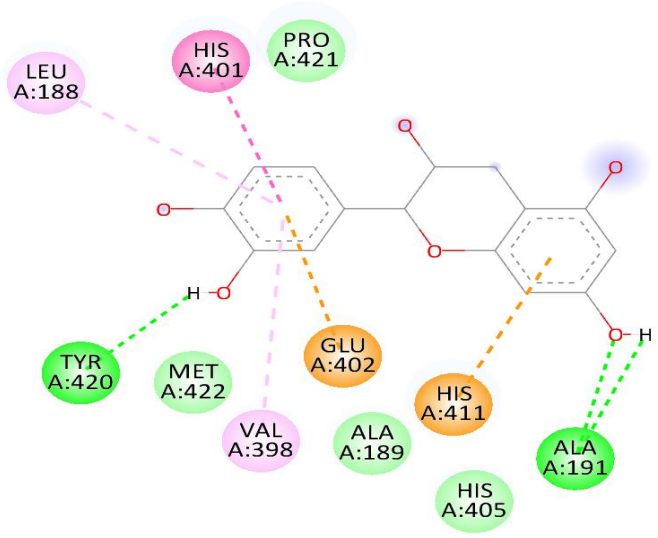


Figure 2: Best Scored Ligands: (+)-Catechin Binding Score = -9.7

2. Interaction between ligands with TGF-β (1PY5). Molecular docking analysis demonstrated that myricetin-3-O-glucoside exhibited a strong binding affinity toward transforming growth factor-beta (TGF-β), with a binding energy of −10.1 kcal/mol. The ligand was found to be well-positioned within the active site, forming a stable ligand–protein complex through multiple non-covalent interactions.

Conventional hydrogen bonding played a key role in stabilizing the complex, with significant interactions observed with residues such as Asp351, Ser280, Leu278, and Ile211. In addition, carbon hydrogen bonds further contributed to binding stability, enhancing the interaction network within the active pocket.

Hydrophobic interactions, including π–alkyl and π–sigma interactions, were identified with residues such as Val219, Leu260, Leu340, and Ala230, which facilitate proper orientation and stabilization of the ligand. Furthermore, several amino acids, including Tyr249, Glu245, Lys337, His283, and Gly286, were involved in van der Waals interactions, supporting overall binding complementarity.

The combination of hydrogen bonding and hydrophobic interactions indicates that myricetin-3-O-glucoside forms a highly stable and energetically favourable complex with TGF-β, suggesting its potential as a promising bioactive compound for modulating TGF-β activity.

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

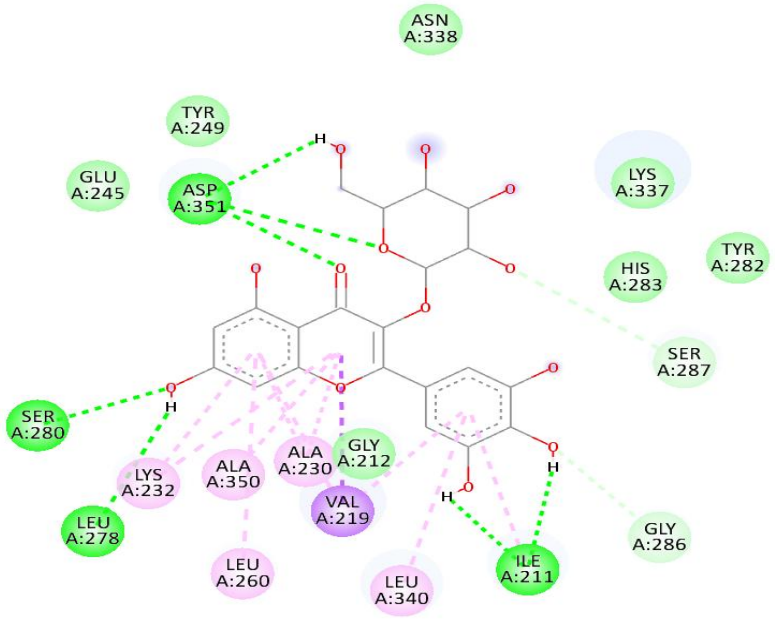


Figure 3: Best Scored Ligand: Myricetin-3-O-glucoside Binding Score = -10.1

3. Interaction between ligands with FGF-2 (1BFB) Molecular docking analysis revealed that rutin exhibited a favourable binding affinity toward fibroblast growth factor-2 (FGF-2), with a binding energy of −7.3 kcal/mol. The ligand was accommodated within the active site and stabilized through a diverse network of non-covalent interactions.

Conventional hydrogen bonds were prominently observed with key residues such as Gln124, Lys120, Val41, and Gly39, which play a crucial role in anchoring the ligand within the binding pocket. Additionally, carbon hydrogen bonding further contributed to the stabilization of the ligand–protein complex.

Electrostatic interactions, including π–anion interaction with Asp42, enhanced the binding affinity by strengthening charge-based interactions. Hydrophobic interactions, such as π–π T-shaped and π–alkyl interactions involving residues like Tyr125, Arg40, and Leu83, were also identified, contributing to proper ligand orientation and stability.

Furthermore, several residues, including Arg34, Gly123, Leu127, Arg82, and Lys126, were involved in van der Waals interactions, supporting the overall structural complementarity between the ligand and the receptor.

Collectively, these interactions suggest that rutin forms a moderately stable complex with FGF-2, indicating its potential as a bioactive compound targeting this protein.

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

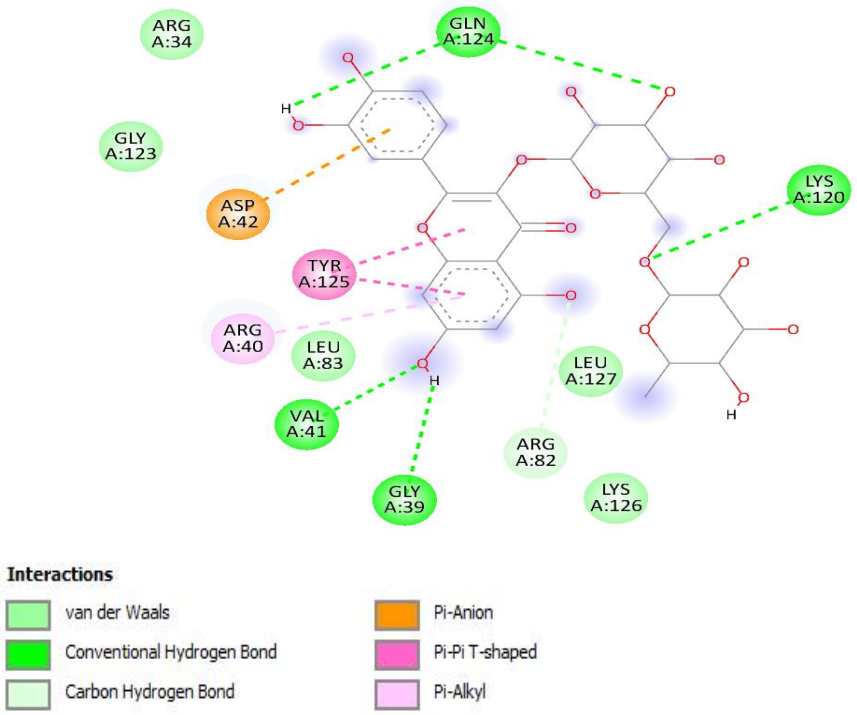


Figure 4: Best Scored Ligand: Rutin Binding Score = -7.3

4.Interaction between ligands with VGEF (3HNG) The molecular docking study was performed to evaluate the binding affinity and interaction patterns of Rutin and Isoquercitrin within the active site of the VEGF protein (PDB ID: 3HNG). The results, as illustrated in Figure 5, demonstrate a strong binding profile with a significant binding score of -8.4 kcal/mol, suggesting high stability and spontaneous binding of the complex.

Key Interaction Findings:

Hydrogen Bonding: The ligands form several conventional hydrogen bonds with key amino acid residues, including ASP 1040, ARG 1021, and HIS 1020. These bonds are crucial for the structural stability of the protein-ligand complex.

Hydrophobic Interactions: Multiple hydrophobic interactions were observed, such as Pi-Alkyl, Pi-Sigma, and Pi-Sulfur interactions involving residues like LEU 1043, LEU 882, and ILE 881. These interactions further stabilize the ligand within the hydrophobic pocket of the receptor.

Van der Waals Forces: A wide range of Van der Waals interactions (e.g., with ALA 874, GLY 1042, and ASN 1027) contribute to the overall binding energy, ensuring a tight fit between the ligands and the protein surface.

Electrostatic Interactions: The presence of Pi-Anion interactions with GLU 878 indicates a diverse bonding environment that enhances the inhibitory potential of these compounds against VEGF.

AMERICAN ACADEMIC PUBLISHER

INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

In conclusion, the high binding affinity and the variety of strong intermolecular forces suggest that Rutin and Isoquercitrin are promising candidates for inhibiting the VEGF signaling pathway, which is a critical target in anti-angiogenic therapy.

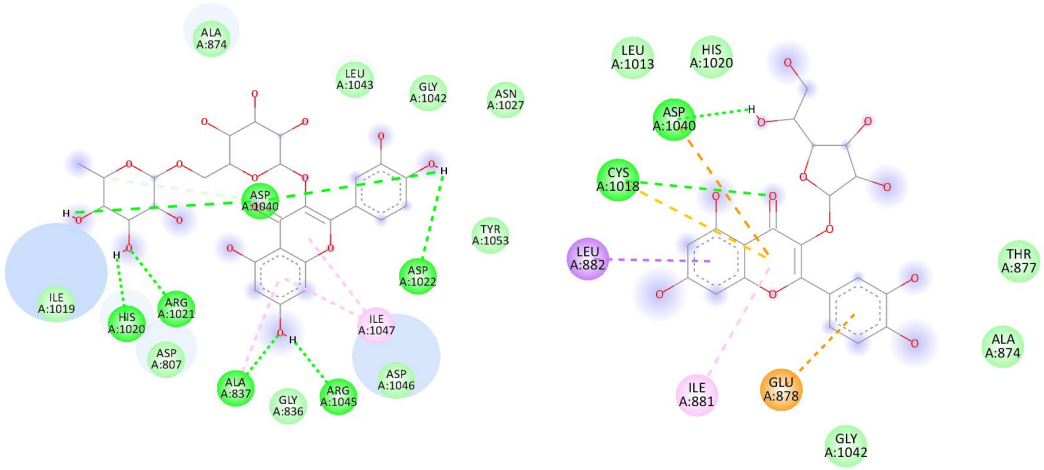


Figure 5: Best Scored Ligands: Rutin, Isoquercitrin Binding Score = -8.4

5. Interaction between ligands with IL-6 (4CNI) The binding orientation and intermolecular interactions of Rutin within the active site of IL-6 (4CNI) were investigated through molecular docking. As depicted in Figure 6, Rutin exhibited a strong binding affinity with a binding score of -8.3 kcal/mol, indicating a stable and thermodynamically favorable protein-ligand complex.

Analysis of Intermolecular Interactions:

Hydrogen Bonding: The stability of the complex is primarily driven by multiple conventional hydrogen bonds. Specifically, Rutin forms strong H-bonds with key amino acid residues, including ASP31, ARG100, and SER102. These bonds are essential for the anchoring of the ligand within the receptor's binding pocket.

Hydrophobic and Pi-Interactions: The aromatic rings of Rutin engage in significant hydrophobic interactions. Pi-Pi Stacked and Pi-Pi T-shaped interactions were observed with residues PHE33 and TYR103, respectively. These interactions contribute to the rigidification of the ligand within the active site.

Van der Waals Forces: A dense network of Van der Waals interactions surrounds the ligand, involving residues such as TYR109, TYR32, SER108, PHE106, GLY105, GLU101, TYR56, and ASN53. These non-covalent forces further enhance the overall binding complementarity.

The combination of strong hydrogen bonding and diverse Pi-interactions underscores the potential of Rutin as a potent inhibitor of IL-6. These findings suggest that Rutin effectively occupies the binding site, which may interfere with IL-6 mediated signaling pathways, often associated with inflammatory responses.

AMERICAN ACADEMIC PUBLISHER

INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

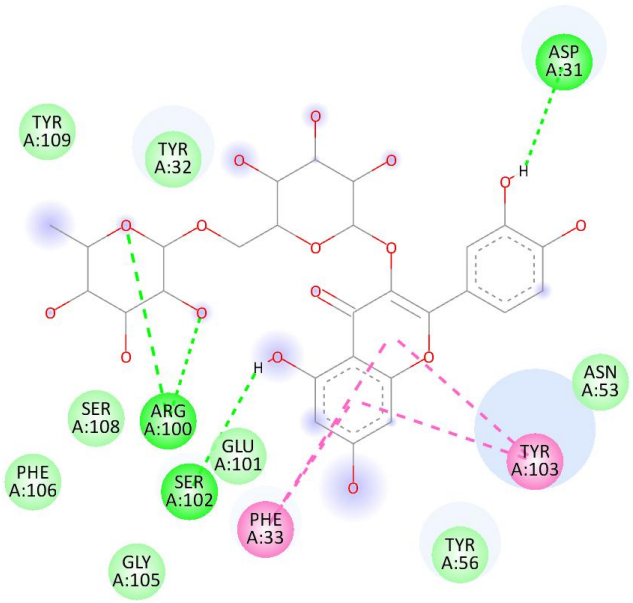


Figure 6: Best Scored Ligand: Rutin Binding Score = -8.3

Conclusion

Finally, we concluded the ethanolic extract of *Calanhoe* Daigremontiana present the flavonoids such as rutin, myricetin-3-O-glucoside, isoquercitrin, caempferol sophoroside, catechin, gallic acid. The combination of hydrogen bonding and hydrophobic interactions indicates that myricetin-3-O-glucoside forms a highly stable and energetically favourable complex with TGF-β, suggesting its potential as a promising bioactive compound for modulating TGF-β activity. Whe concluded the *Calanhoe* Daigremontiana ethanolic extract have excellent wound healing properties. From this docking studies outcome we further plan to in-vitro and in-vivo studies for ethanolic extract.

References

1. Milad R, El-Ahmady S, Singab AN. Genus *Kalanchoe* (Crassulaceae): a review of its ethnomedicinal, botanical, chemical and pharmacological properties. European J Med Plants 2014; 4: 86-104

2. De Araújo E, Félix-Silva J, Xavier-Santos J, Fernándes, J, Guerra GC, de Araújo A, et al. Local anti-inflammatory activity: topical formulation containing Kalanchoe brasiliensis and Kalanchoe pinnata leaf aqueous extract. Biomedicine & Pharmacotherapy. 2019; 113:108721. DOI: 10.1016 / j. biopha.2019.108721 2. Muzitano M, Tinoco L, Guette C, Kaiser C, Rossi-Bergmann B, Costa S. The antileishmanial activity assessment of unusual flavonoids from Kalanchoe pinnata. Phytochemistry. 2006. 67(18):2071–2077.

3. Nabavi S. M., Samec D., Tomczyk M., Milella L., Russo D., Habtemariam S., et al. (2018). Flavonoid biosynthetic pathways in plants: versatile targets for metabolic engineering. *Biotechnol*. Adv. 38:107216. 10.1016/j.biotechadv.2018.11.005

AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES
ISSN NUMBER: 2692 - 5206 Volume 6. No 04. Aprel ,2026

4. Scarano A., Chieppa M., Santino A. (2018). Looking at flavonoid biodiversity in horticultural crops: a colored mine with nutritional benefits. Plants 7: E98. 10.3390/plants7040098
5. Coutinho MA, Muzitano MF, Cruz EA, Bergonzi MC, Kaiser CR, Tinoco LW, Bilia AR, Vincieric FF, Rossi-Bergmann B, Costa SS. Flowers from Kalanchoe pinnata are a rich source of T cell-suppressive flavonoids. Nat Prod Commun. 2012 Feb;7(2):175-8. PMID: 22474947.
6. D. A. Putri and S. Fatmawati, "A New Flavanone as a Potent Antioxidant Isolated from Chromolaena odorata L. Leaves," 2019.
7. J. Eberhardt, D. Santos-Martins, A. F. Tillack, and S. Forli. (2021). AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. Journal of Chemical Information and Modeling.
8. O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 31 (2010) 455-461